FORM PTO-1390 (Modified) (REV 11-98) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: **PU3562USW**

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR): To be assigned 09/787323

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/US99/21092	**14 September 1999**	**16 September 1998**

TITLE OF INVENTION
MULTIPLE SEQUENCING METHOD

APPLICANT(S) FOR DO/EO/US
Burns and Weiner

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ has been transmitted by the International Bureau.
   c. ☒ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ A copy of the International Search Report (PCT/ISA/210).
8. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
9. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
10. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 13 to 20 below concern document(s) or information included:

13. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☒ Certificate of Mailing by Express Mail
20. ☒ Other items or information:

A copy of Request (PCT/RO/101)

Express Mail
EL395941897US

532 Rec'd PCT/PTO 16 MAR 2001

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR 1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
To be assigned 09/787323	PCT/US99/21092	PU3562USW

21. The following fees are submitted:.

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

		CALCULATIONS PTO USE ONLY
☐	Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2) paid to USPTO and International Search Report not prepared by the EPO or JPO	$1,000.00
☐	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but Internation Search Report prepared by the EPO or JPO	$860.00
☐	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	$710.00
☒	International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4)	$690.00
☐	International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4)	$100.00

ENTER APPROPRIATE BASIC FEE AMOUNT =				$690.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).				$0.00	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	10 - 20 =	0	x $18.00	$0.00	
Independent claims	3 - 3 =	0	x $80.00	$0.00	
Multiple Dependent Claims **(check if applicable)**.			☐	$0.00	
TOTAL OF ABOVE CALCULATIONS =				$690.00	
Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) **(check if applicable)**.			☐	$0.00	
SUBTOTAL =				$690.00	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)).			+	$0.00	
TOTAL NATIONAL FEE =				$690.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).**			☐	$0.00	
TOTAL FEES ENCLOSED =				$690.00	
				Amount to be: refunded	$
				charged	$

☐ A check in the amount of to cover the above fees is enclosed.

☒ Please charge my Deposit Account No. **07-1392** in the amount of **$690.00** to cover the above fees.
A duplicate copy of this sheet is enclosed.

☒ The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. **07-1392** A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

23347
PATENT TRADEMARK OFFICE

SIGNATURE

Elizabeth Selby
NAME

38,298
REGISTRATION NUMBER

16 March 2001
DATE

Express Mail
EL395941897US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: Application of: BURNS et al.
International Application No.: PCT/US99/21092
International Filing Date: 14 September 1999
Title: MULTIPLE SEQUENCING METHOD

Honorable Commissioner of Patents
Washington, D.C. 20231

FIRST PRELIMINARY AMENDMENT

Dear Sir:

The above-identified application is being transmitted herewith for entry in the US National Phase under Chapter II of the PCT for the purpose of adding the priority information. Please amend the application as follows:

In the Abstract:

Please substitute for the Abstract the following:

--The present invention provides a method for identifying a nucleic acid utilizing a run-off sequencing reaction of a relatively short portion of the nucleic acid. The method can be utilized, for example, to identify an EST from only a small portion of the EST and in an analysis of nucleotide polymorphisms.--

The Abstract has been placed on a separate sheet of paper according to US practice, as required under 37 CFR 1.72(b).

In the Specification:

On the first line of the specification, after the Title, please add:

--This application is filed pursuant to 35 U.S.C. §371 as a United States National Phase Application of International Application No. PCT/US99/21092 filed 14 September 1999, which claims priority from Provisional Application No. 60/100,491 filed 16 September 1998 and Provisional Application No. 60/100,704 filed 17 September 1998.--

REMARKS

Applicants have attached an abstract on a separate sheet of paper as required by US practice. Applicants have amended the specification for purposes of adding the priority information.

Attached hereto is a marked-up version of the changes made to the specification by the current amendment. The attached page is captioned **"Version with markings to show changes made."** Applicant respectfully requests the entry of the above preliminary amendments.

Examiner is invited and encouraged to contact the undersigned if such contact would facilitate prosecution of this application.

No fee is believed due in connection with this Amendment, however the Commissioner is hereby authorized to charge any under-payment to Deposit Account No. 07-1392.

Respectfully submitted,

Date: 16 March 2001

Elizabeth Selby
Attorney of Record, Reg. No. 38,298

Glaxo Wellcome Inc.
Global Intellectual Property Department
Five Moore Drive, PO Box 13398
Research Triangle Park, NC 27709-3398
Telephone: 919-483-3934
Facsimile: 919-483-7988

CERTIFICATE OF EXPRESS MAILING (37 CFR 1.10)
I hereby certify that this paper (along with any referred to as being attached or enclosed) is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 in an envelope addressed to: Assistant Commissioner of Patents Washington, D.C. 20231 on 3/16/01

Marilyn Eldridge

Version with markings to show changes made

A new paragraph has been added at page 1 immediately after the title.

The abstract has been amended as follows:

The present invention provides a method for identifying a nucleic acid utilizing a run-off sequencing reaction of a relatively short portion of the nucleic acid. The method can be utilized, for example, to identify an EST from only a small portion of the EST and in an analysis of nucleotide polymorphisms. [The figure depicts an untreated and a BpmI-treated sequencing reaction.]

Multiplex Sequencing Method

Background

Field of the Invention

The present invention provides a method for identifying a nucleic acid utilizing a run-off sequencing reaction of a relatively short portion of the nucleic acid. The method can be utilized, for example, to identify an EST from only a small portion of the EST and in an analysis of nucleotide polymorphisms. The reactions can be multiplexed to increase data readout capacity.

Background of the Invention

Several methods have been developed to increase the efficiency of DNA sequencing analysis. These include the methods of i) multiplexing a series of spectrally non-overlapping terminator and/or dye-primer dyes into DNA sequencing lanes, ii) transfer of genomic sequencing reactions to a filter and subsequent hybridization, and iii) multiplex lane-loadings in which 3 instead of 4 sequencing reactions are performed. These methods have mainly been applied to situations in which a long read (greater than several hundred bases *de novo*) is desired.

The present invention is the development of a simple method for multiplexing short sequencing reads (about 16 bases) in the same lane. The application to which we are applying this method is our high-throughput yeast two-hybrid analysis (Buckholz, Stuart, Judelson and Weiner). In this analysis, we desire to sequence short regions of the interacting proteins, and then use a large database to determine the hit identification. Because each bait analyzed generates approximately 100 hits, we needed to develop a method to increase our efficiency of analysis.

Description of the Figures

Figure 1. Untreated and *Bpm*I-treated sequencing reaction. See text for details.

Figure 2. Separation as a function of delta loading time. *Bpm*I-treated PCR fragments were sequenced and multiplexed on the ABI 377 at loadings 1, 2 and 3 at the times indicated post first-loading.

Figure 3. Multiplex loading of a sequencing gel and chromatogram of a single multiplexed lane. Note the chromatogram is not from a lane on the gel shown.

Detailed Description of the Invention

We have developed a method whereby we use reloading of a nucleotide base-calling apparatus, for example polyacrylamide gel electrophoresis or capillary electrophoresis to serial multiplex DNA base-calling. In one example, a run-off sequencing reaction is used to sequence the bases downstream from an endonuclease recognition site. In this method, the endonuclease selected is one that cuts several bases downstream of its recognition site, such that nucleotides from outside the recognition sites would be included in the restricted section of DNA and would then be sequenced in a short-run, run-off sequencing reaction. A short sequencing reaction can be one of 30 or fewer bases, such as 30 bases, 25 bases, 20 bases, 19 bases, 18 bases, 17 bases, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, or 5 bases. For example, in a specific example we term *'BpmI* sequencing,' a run-off sequencing reaction is performed to sequence the 16 bases downstream from a type IIS endonuclease *Bpm*I recognition site.

For this method, a library is constructed wherein the inserts of the library are positioned within the library vector in sufficient proximity to a selected

enzyme recognition site, of an enzyme that cuts downstream of its recognition site, that the insert will be cut by the enzyme within the insert. For example, a library can be constructed from inserts having ligated to them linkers providing the recognition site for the selected enzyme. By way of another example, the vector in which the library is constructed can contain within its multiple cloning site a recognition site for the selected endonuclease to be used to create the template for the run-off sequencing reaction, and the library inserts can be cloned into the vector in a site such that the inserts are in sufficient proximity to the recognition site of the selected enzyme such that the inserts will be cut by the selected enzyme. Furthermore, primers can also be designed to allow amplification of an isolated subclone of the library, prior to performing the restriction and sequencing reactions, wherein the restriction recognition site of the selected endonuclease is retained within the amplified region.

One advantage to this invention is that sequencing reactions can be multiplexed on the analysis apparatus, because it produces short sequences. The sequences determined, however, are sufficient for identifying the isolated nucleic acid by comparison with a sequence database. Thus, for example, two, three, four or more sequences can be run sequentially on the analysis apparatus, allowing for a significant decrease in time and cost of obtaining the data.

One utility of this method is in comparing sequenced cDNA against a cDNA database, for example GenBank. Given such a comprehensive cDNA database, it should be possible to determine the identification of an EST from an analysis of just a small portion of the EST. We are applying this technology to yeast two-hybrid (Y2H) analysis of protein-protein interactions in which a known bait-protein fusion is tested for interactions with an expressed cDNA library. To test the *Bpm*I sequencing method, we cloned randomly primed macrophage cDNA into a yeast two-hybrid cDNA library vector using adapters incorporating a *Bpm*I restriction endonuclease recognition site. Clones have been isolated from the library and tested for the correct gene-call after *Bpm*I sequencing. By sequencing just a small region of DNA adjacent to the cloning site, one can multiplex the DNA sequencing reactions and thereby increase the gene readout capacity of most analytical methods.

Another example of a utility of this invention is in the use of multiplexing sequencing runs applied toward SNP analysis whereby short PCR products containing the region-of-interest are loaded repeatedly into the same well/capillary tube and sequentially-analyzed.

The present method for run-off DNA sequencing can be used to increase the sequencing capacity of a single gel several fold. For example, the *Bpm* I method for run-off DNA sequencing can be used to increase the sequencing capacity of a single gel at least 4 fold. A 16 bp read from one end of the clone can be used to correctly identify many clones. With the implementation of Bioinformatics tools such as sample tracking software and a tool to merge the BLAST results of the forward and reverse reactions, this methodology can be used to support Y2H in a higher-throughput environment.

The enzyme utilized to cut the nucleic acid sample for sequencing is an enzyme that cuts at least 1 base downstream of its recognition site, so that the run-off sequencing event produces sequence data including the nucleotide sequences of the library insert up to the point of restriction by the enzyme. Thus, the enzyme can be a restriction endonuclease. In addition to *Bpm* I, exemplified herein, which cuts 16 bases downstream of its recognition site, other non-palindromic endonucleases such as *Bsg* I (16/14) and *Eco*57 I (16/14) can readily be used to design linkers for run-off sequencing. For further example, *Bcg* I, *Fok* I, or another enzyme which would allow a longer read, *Mme* I (20/18), could be utilized. The enzyme can be chosen by considering the number of bases of sequence data desired for the specific purpose.

Additional optimization of this technology can be done. Redesign of sequencing primers to read closer to the cloning site will allow for shorter sequencing reads and increase the multiplexing capacity of the gel. Additionally, longer run times on the ABI 377XL may have an advantage. Furthermore, a system featuring automated sample loading, such as the ABI 310 can be utilized.

Analysis may be performed by any means desired. For example, analysis of gel electrophoresis, analysis on a capillary apparatus, or analysis by mass spectrophotometry can be performed.

Also provided is a kit for performing multiplex analysis of sequencing reactions comprising: an enzyme that cuts at least 1 base downstream of a selected enzyme recognition site; and a set of oligonucleotide linkers comprising a recognition site for the selected enzyme. For example, the enzyme can be *Bpm I, Bsg I, Eco57,*or *MmeI* or a combination thereof. The kit can further comprise, for example, a vector for constructing a library wherein, for example, the vector has an appropriate cloning site for use in the method. The kit can further comprise a component to facilitate the multiplexing of the sequence reaction products, selected according to the analysis method to be used.

Examples

cDNA library construction. Polyadenylated RNA was isolated from 5 x 10^7 THP1 cells using FastTrack 2.0 (Invitrogen, San Diego, CA). A random oligomer primed cDNA library was constructed from 5 μg of the polyA-selected mRNA using the Copy Kit (Invitrogen). *E. coli* DNA ligase was removed from the second-strand synthesis reaction to enhance synthesis of products approximately 900 base pairs in length. Next, *Bpm*I linkers (5'-AATTCGGCTCGAGCTGGAG-3' and 5'-CTCCAGCTCGAGCCG-3') were added to the ends of the blunt-ended cDNA fragments using T4 DNA ligase. Following the addition of the linkers, the fragments were phosphorylated (T4 DNA kinase) and size selected using a Chromaspin 400 column (Clontech, Palo Alta, CA). The cloning vector pYesTrp2 (Invitrogen) was digested using the restriction endonuclease *Eco*RI at 37 °C. The linearized vector was dephosphorylated with shrimp alkaline phosphatase (SAP, Boerhinger Mannheim) prior to gel purification. cDNA inserts and treated, linearized vector DNAs were ligated into the cloning vector and the ligation product was transformed into

Electromax DH10B competent cells (Life Technologies Inc., Gaithersburg, MD). Colonies were selected on LB agar plates with ampicillin.

***Bpm*I sequencing.** Plasmid DNAs were isolated using the R.E.A.L prep (Qiagen, Valencia, CA). One μg of plasmid DNA was digested with 2 U of *Bpm*I (New England Biolabs, Beverly, MA) for at least two hours at 37 °C. Reactions were precipitated with sodium acetate and ethanol, pelleted for 30 min at 3K RPM in a Sorvall RC3B centrifuge rotor. The supernatants were decanted and the pellets were washed with 70% ethanol and dried prior to preparation of sequencing reactions. Using standard conditions, 500 ng of digested DNA was cycle-sequenced using 3.2 pM of primer pYesTrpF or pYesTrpR (Invitrogen) and Big Dye Terminators (PE Biosystems, Foster City, CA). Excess primers and nucleotides were removed using a gel filtration cartridge (Edge Biosystems, Gaithersburg, MD). Products were analyzed on either an ABI 377 or ABI 310 automated sequencer under conditions as specified by the manufacturer and subjected to BLAST analysis against the GenBank database (Table 1).

Table 1. Blast Results

	Undigested			Digested (100% match)	
Clone[a]	Gene Call	E-val	5'-16 Bases	3'-16 Bases
1	Rattus norvegicus RNA helicase	6e-32	--	Didn't cut
6	H. sapiens PAC clone DJ0170019 from 7p15-p21	3e-27	--	--
8	H. sapiens Pig8 mRNA	e-127	+	--
14	H. sapiens mRNA for hnRNPcore protein A1	e-111	+	+
15	Rattus norvegicus unc-50 related protein mRNA	1e-54	+	polyA+[b]
16	Human calmodulin-dependent protein phosp.	e-127	+	+
17	H. sapiens DNA sequence from BAC217C2	e-133	+	+
18	H. sapiens mRNA for putative DNAmethyltrans.	e-140	+	polyA+
21	H. sapiens splicing factor Sip1 mRNA	e-138	+	--
22	Human DNA sequence from cosmid N114B2	2.5	--	--
27	H. sapiens chromosome 16 BAC clone	1e-27	+	polyA+
32	H. sapiens PAC clone DJ0777023 from 7p14-p15	1e-94	--	--
35	Human DNA sequence from PAC417G15	1e-08	--	--
36	Human alpha satellite DNA	4e-39	+	polyA+
37	H. sapiens DNA sequence from BAC 747E2	1e-48	--	--
44	H. sapiens homolog of Nedd5 mRNA	7e-84	+	poor qual. seq.
47	Human kidney mRNA for catalase	e-122	+	+
48	Human DNA sequence "sequence in progress"	4e-83	--	--

[a] mitochondrial (16%), polyA+ only (4%) & cloning vector (2%) hits eliminated

[b] end contained only polyA+ sequence

What is claimed is:

1. A method of identifying a nucleic acid comprising performing gel or capillary electrophoresis on a series of two or more short sequencing reaction products loaded sequentially onto the same lanes of a sequencing gel, a first sequencing reaction product being loaded at a first loading time and a second short sequencing reaction product being loaded at a second loading time, wherein the first loading time and the second loading time are sufficiently temporally separated to separate the first sequencing reaction product from the second sequencing reaction product by electrophoresis.

2. The method of claim 1, wherein the sequencing reaction product is produced from a region comprising a SNP (single nucleotide polymorphism).

3. The method of claim 1, wherein the sequencing reaction product is produced from an EST (expressed sequence tag).

4. The method of claim 1, wherein the short sequencing reaction products are about 20 bases or shorter.

5. The method of claim 1, wherein the short sequencing reaction products are run off sequencing reaction products.

6. A method of determining the nucleotide sequence of a selected portion of a nucleic acid comprising:

a) isolating the nucleic acid from a nucleic acid library wherein the library comprises a recognition site of a selected enzyme that cuts at least 1 base downstream of the recognition site, wherein the recognition site is positioned within 1 base of the inserts of the library;

b) amplifying the nucleic acid;

c) digesting the amplified nucleic acid with the selected enzyme;

d) performing a run-off sequencing reaction utilizing a primer that hybridizes to a region of the amplified fragment at or upstream of the recognition site to form a first sequencing reaction product; and

e) analyzing the first sequencing reaction product.

7. The method of claim 6, wherein a second sequencing reaction product is analyzed sequentially on the same analysis run as the first sequencing reaction product.

8. The method of claim 6, wherein the selected enzyme is a restriction enzyme.

9. The method of claim 8, wherein the selected restriction enzyme is *BpmI*.

10. The method of claim 6, wherein the analysis performed is gel electrophoresis.

11. The method of claim 6, wherein the analysis is performed with a capillary apparatus.

12. The method of claim 6, wherein the analysis performed is mass spectrophotometry.

13. A kit for performing multiplex analysis of sequencing reactions comprising:

a) an enzyme that cuts at least 1 base downstream of a selected enzyme recognition site; and

b) a set of oligonucleotide linkers comprising a recognition site for the selected enzyme.

ABSTRACT OF THE DISCLOSURE

Multiple Sequencing Method

The present invention provides a method for identifying a nucleic acid utilizing a run-off sequencing reaction of a relatively short portion of the nucleic acid. The method can be utilized, for example, to identify an EST from only a small portion of the EST and in an analysis of nucleotide polymorphisms.

COMBINED DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION WITH POWER OF ATTORNEY

ATTORNEY'S DOCKET PU3562USW
First Names Inventor: **Burns**
Complete if known: App No.:
Filing Date Concurrently herewith
Group Art Unit:

(X) Declaration submitted with initial filing or

( )Declaration submitted after initial filing (surcharge required 37CFR1.16(e))

As below named inventor. I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

MULTIPLE SEQUENCING METHOD

the specification of which (check only one item below):

[ ]is attached hereto.
OR
[X] was filed on _____as United States application Serial No. _____ or **PCT** International

Application Number ___PCT/US99/21092___ filed 14 September 1999 and was amended on (MM/DD/YYYY) _____(if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR §1.56.

I hereby claim foreign priority benefits under 35, U.S.C. §119 (a)-(d) or §365(b) of any foreign applications(s) for patent or inventor's certificate or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or of any PCT international application having a filing date before that of the application on which priority is claimed:

PRIOR FOREIGN AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. 119:

Prior Foreign Application Number (s)	Country	Foreign Filing Date (MM/DD/YYYY))	PRIORITY CLAIMED
1.			
2.			
3.			
4.			
5.			

I hereby claim the benefit under Title 35, United States Code §119(e) of any United States provisional application(s) listed below:

Application No.	Filing Date (MM/DD/YYYY)	
1. 60/100,491	09/16/1998	
2. 60/100,704	09/17/1998	
3.		
4.		
5.		

COMBINED DECLARATION FOR UTILITY or DESIGN PATENT APPLICATION WITH POWER OF ATTORNEY Continued

ATTORNEY'S DOCKET NUMBER: PU3562USW

I hereby claim the benefit under 35, U.S.C. §120 of any United States application or §365(c) of any PCT international application designating the United States of America that is listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 C.F.R. §1.56 which became available between the filing date of the prior application(s) and the national or PCT international filing date of this application:

PRIOR U.S. PARENT APPLICATION or PCT PARENT APPLICATION

U.S. Parent Application or PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	STATUS (Check one) PATENTED	PENDING	ABANDONED

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the U.S. Patent and Trademark Office connected therewith. (List name and registration number)

David J. Levy	Reg. No. 27,655	James P. Riek	Reg. No. 39,009	Bonnie L. Deppenbrock	Reg. No. 28,209
Charles E. Dadswell	Reg. No. 35,851	Virginia C. Bennett	Reg. No. 37,092	John L. Lemanowicz	Reg. No. 37,380
Karen L. Prus	Reg. No. 39,337	Frank P.Grassler	Reg. No. 31,164		
Robert H. Brink	Reg. No. 36,094	Christopher P. Rogers	Reg. No. 36,334		
Elizabeth Selby	Reg. No. 38,298	Lorie Ann Morgan	Reg. No. 38,181		

Send Correspondence to:
David J. Levy, Patent Counsel
Global Intellectual Property Department
Glaxo Wellcome Inc.
Five Moore Drive, PO Box 13398
Research Triangle Park, NC 27709

23347
PATENT TRADEMARK OFFICE

Direct Telephone Calls to:
Elizabeth Selby
919-483-3934

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

2	FULL NAME OF INVENTOR	FAMILY NAME: BURNS	FIRST GIVEN NAME: Daniel	SECOND GIVEN NAME/INITIAL: Keith
100	INVENTOR'S SIGNATURE	Daniel K Burns		DATE: 16 March 2001
0	RESIDENCE & CITIZENSHIP	CITY: Apex	STATE OR FOREIGN COUNTRY: NC NC	COUNTRY OF CITIZENSHIP: US
1	POST OFFICE ADDRESS	POST OFFICE ADDRESS: C/o Glaxo Wellcome Inc. Five Moore Drive, PO 13398	CITY: Research Triangle Park	STATE & ZIP CODE/COUNTRY: NC 27709 US
2	FULL NAME OF INVENTOR	FAMILY NAME: WEINER	FIRST GIVEN NAME: Michael	SECOND GIVEN NAME/INITIAL: Phillip
	INVENTOR'S SIGNATURE			DATE:
0	RESIDENCE & CITIZENSHIP	CITY: Cary	STATE OR FOREIGN COUNTRY: NC	COUNTRY OF CITIZENSHIP: US
2	POST OFFICE ADDRESS	POST OFFICE ADDRESS: 102 Spring Bud Drive	CITY: Cary	STATE & ZIP CODE/COUNTRY: NC 27513 US
2	FULL NAME OF INVENTOR	FAMILY NAME	FIRST GIVEN NAME	SECOND GIVEN NAME/INITIAL
	INVENTOR'S SIGNATURE			DATE:
0	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
3	POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE & ZIP CODE/COUNTRY

COMBINED DECLARATION FOR UTILITY or DESIGN PATENT APPLICATION WITH POWER OF ATTORNEY Continued

ATTORNEY'S DOCKET NUMBER: **PU3562USW**

I hereby claim the benefit under 35, U.S.C. §120 of any United States application or §365(c) of any PCT international application designating the United States of America that is listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U S C. §112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 C.F.R. §1.56 which became available between the filing date of the prior application(s) and the national or PCT international filing date of this application:

PRIOR U.S. PARENT APPLICATION or PCT PARENT APPLICATION

U.S. Parent Application or PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	STATUS (Check one) PATENTED	PENDING	ABANDONED

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the U.S. Patent and Trademark Office connected therewith. (List name and registration number)

David J. Levy	Reg. No. 27,655	James P Riek	Reg. No. 39,009	Bonnie L. Deppenbrock	Reg. No. 28,209
Charles E. Dadswell	Reg No. 35,851	Virginia C. Bennett	Reg. No. 37,092	John L. Lemanowicz	Reg No 37,380
Karen L. Prus	Reg. No 39,337	Frank P.Grassler	Reg No. 31,164		
Robert H. Brink	Reg. No. 36,094	Christopher P. Rogers	Reg. No. 36,334		
Elizabeth Selby	Reg. No. 38,298	Lorie Ann Morgan	Reg. No. 38,181		

Send Correspondence to:
David J. Levy, Patent Counsel
Global Intellectual Property Department
Glaxo Wellcome Inc.
Five Moore Drive, PO Box 13398
Research Triangle Park, NC 27709

23347
PATENT TRADEMARK OFFICE

Direct Telephone Calls to
Elizabeth Selby
919-483-3934

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

FULL NAME OF INVENTOR	FAMILY NAME: **BURNS**	FIRST GIVEN NAME: **Daniel**	SECOND GIVEN NAME/INITIAL: **Keith**
INVENTOR'S SIGNATURE			**DATE:**
RESIDENCE & CITIZENSHIP	CITY: **Durham**	STATE OR FOREIGN COUNTRY: **NC**	COUNTRY OF CITIZENSHIP: **US**
POST OFFICE ADDRESS	POST OFFICE ADDRESS: **C/o Glaxo Wellcome Ine. Five Moore Drive, PO 13398**	CITY: **Research Triangle Park**	STATE & ZIP CODE/COUNTRY: **NC 27709 US**
FULL NAME OF INVENTOR	FAMILY NAME: **WEINER**	FIRST GIVEN NAME: **Michael**	SECOND GIVEN NAME/INITIAL: **Phillip**
INVENTOR'S SIGNATURE			**DATE:** 03 07 01
RESIDENCE & CITIZENSHIP	CITY: **Cary**	STATE OR FOREIGN COUNTRY: **NC** NC	COUNTRY OF CITIZENSHIP: **US**
POST OFFICE ADDRESS	POST OFFICE ADDRESS: **102 Spring Bud Drive**	CITY: **Cary**	STATE & ZIP CODE/COUNTRY: **NC 27513 US**
FULL NAME OF INVENTOR	FAMILY NAME	FIRST GIVEN NAME	SECOND GIVEN NAME/INITIAL
INVENTOR'S SIGNATURE			**DATE:**
RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE & ZIP CODE/COUNTRY